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Charles B. Templeton, Mark A. Poli and Ross D. LeClaire

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The cardiorespiratory effects of various doses of brevetoxin (0 - 100 µg PbTx-2/kg) were studied in conscious, tethered rats. After surgical preparation and a 24 hr recovery, toxin or vehicle was infused into the tethered, awake rats for 1 hr. They were then monitored for 6 hr or until death. Toxin-infused rats had decreased core and peripheral temperatures and decreased respiratory rates; these values were all low in the 100 µg/kg group at the time of death. Blood gas values remained within normal limits, except terminally. Electrocardiographic (ECG) disturbances, noted in all groups given ≥25 µg/kg, included heart block, premature ventricular contractions and idioventricular rhythms. It was concluded that brevetoxin causes changes in cardiac conduction and multiple changes in nervous system function.

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CARDIORESPIRATORY EFFECTS OF BREVETOXIN (PbTx-2) IN CONSCIOUS, TETHERED RATS

CHARLES B. TEMPLETON, MARK A. POLI,
AND ROSS D. LECLAIRE

Pathophysiology Division
U.S. Army Medical Research Institute of Infectious Diseases
Fort Detrick, Frederick, MD. 21701-5011, U.S.A.

Correspondence: Charles B. Templeton, DVM, PhD
c/o Pathophysiology Division
U.S.A.M.R.I.I.D., Fort Detrick
Frederick, Maryland 21701-5011, U. S. A.

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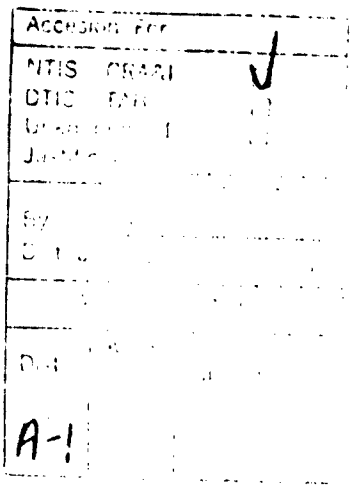
The opinions of the authors in no way reflect the opinions of the Department of the Army or the Department of Defense.



C. B. Templeton, M. A. Poli, and R. D. LeClaire. Cardiorespiratory effects of brevetoxin (PbTx-2) in conscious, tethered rats. Toxicon __, ___-___, 198_. - The cardiorespiratory effects of various doses of brevetoxin (0 - 100 ug PbTx-2/kg) were studied in conscious, tethered rats. After surgical preparation and a 24 hr recovery, toxin or vehicle was infused into the tethered, awake rats for 1 hr. They were then monitored for 6 hr or until death. Toxin-infused rats had decreased core and peripheral temperatures and decreased respiratory rates; these values were all low in the 100 ug/kg group at the time of death. Blood gas values remained within normal limits, except terminally. Electrocardiographic (ECG) disturbances, noted in all groups given $\geq$25 ug/kg, included heart block, premature ventricular contractions and idioventricular rhythms. It was concluded that brevetoxin causes changes in cardiac conduction and multiple changes in nervous system function.

INTRODUCTION

Brevetoxins are cyclic polyether compounds produced by the marine dinoflagellate, Ptychodiscus brevis [NAKANISHI, 1986]. Blooms of P. brevis are responsible for massive fish kills during red tide occurrences around Florida and in other coastal waters (DAVIS 1947). Neurotoxins in sea spray cause ocular and respiratory irritation, and consumption of seafood containing brevetoxin causes neurotoxic shellfish poisoning (BADEN 1983).

At least eight active brevetoxins (PbTx-1 through PbTx-8) have been isolated from P. brevis cell cultures [POLI et al., 1986]. Toxic fractions produce diverse physiological effects in vivo and have been studied in a number of in vitro nerve and muscle tissue preparations (BADEN 1983; STEIDINGER and BADEN, 1984). The site of action of PbTx-2 and PbTx-3 is the voltage-sensitive sodium channel (HUANG et al., 1984; POLI et al., 1986). The compounds bind to a specific receptor site on the channel complex where they cause persistent activation, increased Na+ flux, and subsequent depolarization of excitable cells at resting membrane potentials (POLI et al., 1986).

Among the physiological changes reported in anesthetized, spontaneously breathing dogs given 15-120 ug/kg brevetoxin i.v. are periods of apnea and respiratory arrest, hypertension and hypotension, skeletal muscle fasciculations and cardiac arrhythmias (JOHNSON et al., 1985). Under conditions of pentobarbital anesthesia and controlled end-expiratory Pco_2, cats injected with brevetoxin at a dose rate of 40 ug/kg i.v. display the Bezold-Jarish reflex triad of bradycardia, hypotension and bradypnea, which is abolished by vagotomy (BORISON et al., 1985). In both of these studies, signs were reduced or abolished by use of cholinergic antagonists. In other studies, dogs given 20 to 80 ug/kg brevetoxin i.v. showed ECG abnormalities, transient apnea, and, at high doses (100 ug/kg i.v.), ventricular fibrillation refractory to cardiac massage and electro-version (ELLIS et al., 1979). Brevetoxin-induced bronchoconstriction in the guinea pig is antagonized by atropine but not by transection of the vagus nerve (BADEN and MENDE, 1982).

The purpose of this study was to examine pathophysiological changes associated with brevetoxin (PbTx-2) toxicity in the conscious, tethered rat. We chose the awake model to avoid anesthesia, which may alter the neurological and behavioral responses seen in PbTx-2-treated rats.

MATERIALS AND METHODS

Male rats (CRL CD SDBR, Charles River Laboratories, Wilmington, MA 01887; 350-500 g) were anesthetized with 55 mg/kg pentobarbital i.p. and placed on a heated surgical board (Harvard Apparatus Co., Inc., South Natick, MA 01760). A catheter (PE50, Clay-Adams, Parsipany, NJ 07054), placed into the carotid artery and advanced until the distal tip resided in the aorta, was used to measure arterial blood pressure and to sample blood for blood gas measurements. Another catheter (PE50), placed into the jugular vein and advanced until the distal tip was near the cranial vena cava, was used for toxin infusion. Thermistor probes (Sensortek, Clifton, NJ 07013) were implanted into the abdominal cavity and s.c. over the sternum. Teflon-coated, stainless steel electrocardiograph (ECG) leads were placed s.c. over the ventral and dorsal thorax to obtain a V10 tracing. All lead wires and catheters were tunneled s.c. to the dorsal cervical area and passed through a 20 cm steel spring tether (Alice Chatham King, Carmel, CA 90043). Catheters were flushed with heparinized saline and plugged with stainless steel pins. The animals were placed in cages and their tethers passed through the wire mesh cage top to allow sampling and monitoring. After recovery from

anesthesia, rats were able to move freely about the cage while being monitored; sampling was conducted without perturbing the animals.

To characterize the responses to PbTx-2 (Daniel Baden, Univ. of Miami, Miami, FL 33149), five dose rates (0, 12.5, 25, 50, and 100 ug/kg) were infused (Harvard Apparatus Co., Inc., South Natick, MA 01760; 2 ml/hr) for 1 hr into the jugular catheters of rats (four rats per group). Vehicle for the toxin consisted of 2 ml saline containing 0.5% emulsifier (Emulphor EL-620, GAF Corp., New York, NY 10020). Control (0 ug/kg) rats were given vehicle only. Heart rates, systolic and diastolic arterial blood pressures, pulse pressures, core and peripheral body temperatures and lead V10 ECG's were monitored continuously (Gould, Inc., Cleveland, OH 44114). Respiratory rates were counted and recorded and blood gases were determined every 15 min for 3 hr, then each 30 min for the remainder of the study (ILS1306, Instrument Laboratory Systems, Inc., Lexington, MA 02173). Clinical signs and behavior were recorded in selected animals by video camera. After infusion, monitoring continued for an additional 5 hr, by which time most rats had died or measured parameters had returned to near baseline levels.

Two types of analysis were performed on each measured

parameter. First, a profile analysis was performed using an Analysis of Variance. The second analysis involved multiple comparisons of all time points to baseline or time 0. The LD_{50} was calculated after the method of WEIL (1952).

RESULTS

All four rats infused with 100 ug/kg PbTx-2 died within 2 hr. One of the four rats infused with 50 ug/kg died during the 6 hr study. All other animals survived. The calculated 24 hr LD_{50} for this study of 16 animals given a 1 hr intravenous infusion of PbTx-2 was 60 ug/kg.

The respiratory rates of the three highest dose groups fell to 20% of baseline within 90 min ($p < 0.01$, Fig. 1). The 12.5 ug/kg group mean fell to near 60% of baseline during this time. Except for the terminal values of the high-dose group, blood gas analyses showed that Po_2, Pco_2, pH, HCO_3, base excess and total CO_2, remained within normal limits during the entire 6 hr study ($p > 0.05$, data not shown). The 100 ug/kg group typically displayed evidence of hypoventilation terminally, namely, hypercarbia, acidosis and low oxygen tension. Clinically, the animals appeared to ventilate very deeply. At 6 hr, all survivors had respiratory rates within the normal range except the 50 ug/kg group, which had recovered to only about 60% of baseline.

Each treatment group showed a dose-dependent decrease in core body temperature (Fig. 2) and all but the 12.5 ug/kg

group showed a significant decrease in peripheral body temperature (Fig. 3) ($p < 0.01$). The three highest dose groups decreased 3-4°C in core and 2-3.5°C in peripheral temperature. The decrease in the lowest dose group averaged 0.5°C. In all groups, the temperature decrease occurred during the first 2 hr. In the 50 ug/kg group, survivors showed no significant recovery during the 6 hr study, but were near normal after 24 hr.

Numerous cardiac arrhythmias were noted, including premature ventricular depolarizations, transient idioventricular rhythms and complete heart block (Fig. 4). Mean heart rate was not significantly altered, however, except in the 50 and 100 ug/kg groups (data not shown). Systolic and diastolic arterial blood pressures did not change significantly in any group ($p > 0.05$), but pulse pressures increased in the two highest-dose groups ($p < 0.01$, data not shown). Bradycardia and hypotension were seen in the 100 ug/kg group just before death, when the rats showed signs of complete cardiorespiratory collapse.

Rats infused with PbTx-2 exhibited numerous and varied clinical signs. Consistent in all rats were gasping-like abdominal movements, head-bobbing, depression, loss of righting reflexes, and ataxia. Time of onset varied, but depression and gasping movements were generally the first

clinical manifestations of intoxication. Head-bobbing and ataxia usually began 2-3 hr after infusion. In some rats, apparently uncontrolled muscular contractions occurred, usually in the hindquarters, causing the animals to lunge violently across the cage in one coordinated motion. Neither behavior nor measured physiological parameters were altered in control animals.

DISCUSSION

The most consistent response observed was a rapid fall in respiratory rates, occurring shortly after the beginning of toxin infusion. The decrease was precipitous and dose-dependent; however, the rats ventilated very deeply, suggesting compensation by increased tidal volumes. In studies with cats given an accumulated dose of 280 ug/kg brevetoxin, central CO_2 responsiveness was still present even when respiratory rates decreased to as low as 20 breaths/min (BORISON et al., 1980). This suggests that the ability to compensate for decreased respiratory rate is still intact. Normal blood gas values support this conclusion. Decreased respiratory rates and respiratory arrests have been reported in dogs (ELLIS 1979, JOHNSON et al., 1985), and cats (BORISON et al., 1980, BORISON et al., 1985). In all these studies, respiratory rate dysfunction

was the hallmark of toxicity and respiratory failure the cause of death.

Simultaneous decreases in core and peripheral body temperatures began almost immediately after the start of the infusion period. The temperature decrease was dose-dependent and correlated well with the onset of listlessness and recumbency. All groups reached their lowest temperature by 120 min, but only the two highest-dose groups failed to become normothermic by 6 hr. Reduced body temperatures could reflect decreased metabolic rate and reduced heat production, or may simply be a result of peripheral vasodilation.

Arrhythmias recorded by lead V10 ECG indicated conduction defects in the Purkinje system of the heart. These arrhythmias were typically seen late in the 6 hr monitoring period and were most common in the higher-dose groups. Arrhythmias were also consistently observed in the previously mentioned studies using dogs and cats. Additionally, rat and guinea pig hearts treated with 1.25×10^{-8} and 1.87×10^{-7}M brevetoxin consistently developed arrhythmias characterized by ventricular tachycardia and A-V block (RODGERS et al., 1984). The maintenance of heart rates and systolic and diastolic pressures, concomitant with the increase in pulse pressures, suggested compensation for

decreased vascular resistance by an increased cardiac output (BRAUNWALD, 1980).

Behavioral changes and neurological signs were similar in all groups of toxin-infused rats. At the highest dose (100 ug/kg), rats became dorsally and laterally recumbent after about 3 hr. Loss of the placement reflex suggests pathology of spinal origin, while taxis and convulsive movements may have originated in the brain stem. At times, both hindquarters contracted simultaneously, indicating the initiating impulse originated at the level of the spinal cord or higher. This motion, and head-bobbing, are indicative of dysfunction of fine motor movement suggestive of cerebrospinal tract involvement and somatomotor seizures. Somatomotor seizures have been previously reported in cats given 160 ug/kg brevetoxin i.v. (BORISON et al., 1985). These signs, coupled with decrease in core temperature, suggest a more central effect, and more specifically, the brain stem and cerebellum. This conclusion was supported in later studies when several animals developed head-tilt, a condition highly suggestive of central nervous system involvement.

In summary, i.v. infusion of PbTx-2 caused toxic signs indicative of nervous system involvement, including a precipitous fall in core and peripheral body temperatures

and clinical signs of dysfunction that might have originated in the brain stem or the spinal cord. The profound decreases in respiratory rates could also be mediated through peripheral reflex mechanisms. Compensation for these respiratory rate changes in the surviving animals occurred to the extent that blood gases and blood pressures remained essentially normal.

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FIGURE LEGENDS

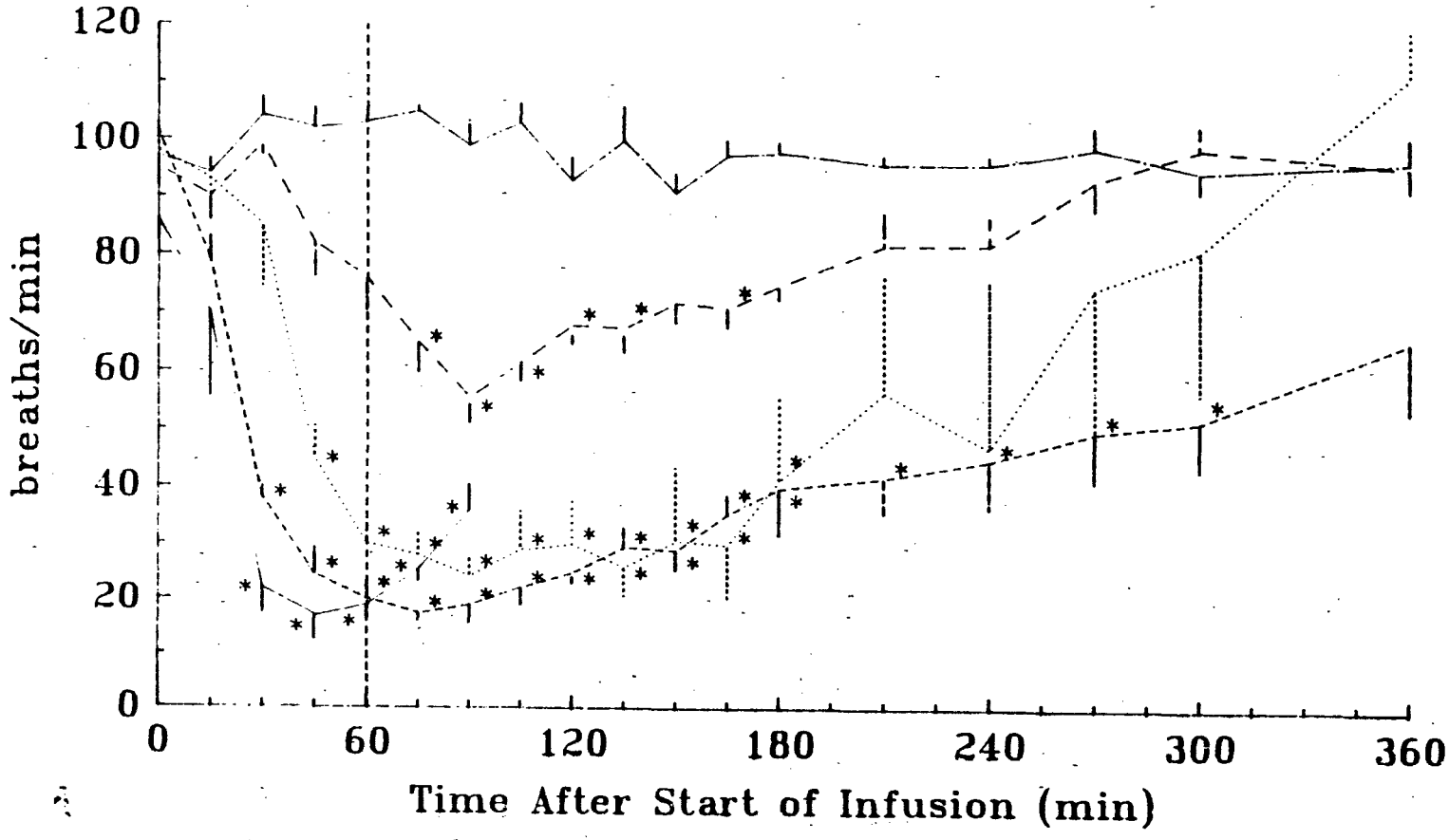
Figure 1. Dose-response curve showing effect of 1 hr infusion of PbTx-2 on respiratory rate in rats. [solid = 100 ug/kg; dashed = 50 ug/kg; dotted = 25 ug/kg; long dashed = 12.5 ug/kg; dot/dashed = 0 ug/kg.] Vertical dash represents end of infusion period. Symbols represent points significantly different from baseline ($p < 0.01$).

Figure 2. Dose-response curve showing effect of 1 hr infusion of PbTx-2 on core temperature in rats. [solid = 100 ug/kg; dashed = 50 ug/kg; dotted = 25 ug/kg; long dashed = 12.5 ug/kg; dot/dashed = 0 ug/kg.] Symbols represent points significantly different from baseline ($p < 0.01$).

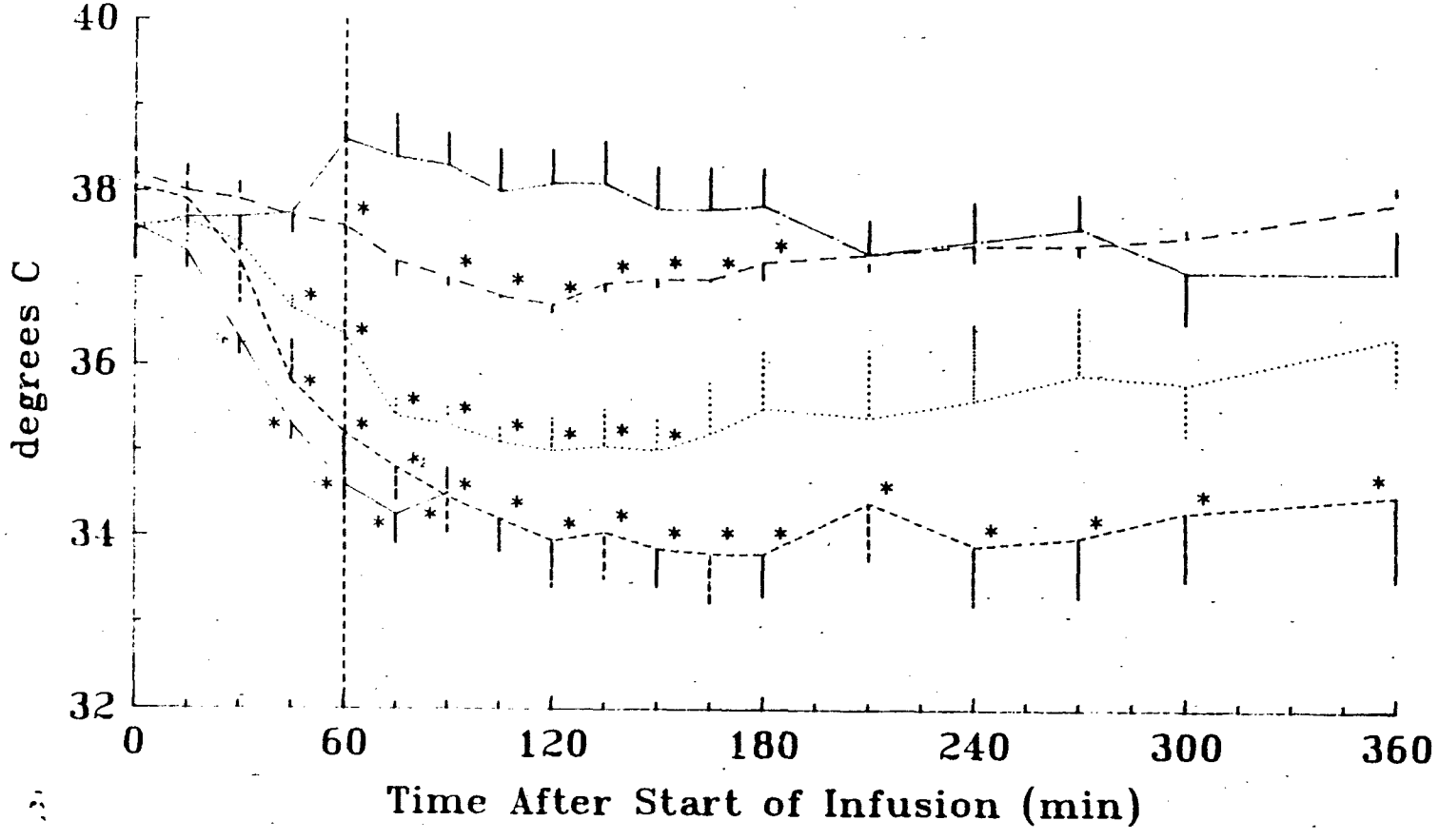
Figure 3. Dose-response curve showing effect of 1 hr infusion of PbTx-2 on peripheral temperature in rats. [solid = 100 ug/kg; dashed = 50 ug/kg; dotted = 25 ug/kg; long dashed = 12.5 ug/kg; dot/dashed = 0 ug/kg.] Symbols represent points significantly different from baseline ($p < 0.01$).

Figure 4. Lead II electrocardiograms showing effect of 1 hr infusion of PbTx-2 (50 ug/kg) on cardiac rhythms in rats. From top: premature ventricular depolarizations, idioventricular rhythm, complete heart block and normal rat ECG. (1 cm/mV, 25 mm/sec).

Respiratory Rate



Core Body Temperature



Peripheral Body Temperature